

The Study of Milk Containing *Lactobacillus Acidophilus* on Histological and Serum Markers of Liver Tissue Injury in Streptozotoc-3

A. Ghorbani Ranjbary (DVM)*¹, R. Bahadori (MD)¹, R. Moridi (MD)¹

1. Young Researchers and Elite Club, Kazerun Branch, Islamic Azad University, Kazerun, I.R.Iran

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ABSTRACT

BACKGROUND AND OBJECTIVE: Diabetes is known as one the leading causes of hepatic disorders. The aim of this study was to assess the liver damage in diabetes mellitus and the protective effect of milk containing *Lactobacillus acidophilus* (LA) in rats with diabetes induced by streptozotocin.

METHODS: In this experimental study, 60 male Wistar rats were randomly divided into 6 groups including: control diabetic, normal treated with 10 and 20% milk containing LA, and diabetic rats treated with 10 and 20 percent of milk containing LA. After 60 days fasting, blood samples were obtained directly from cardiac puncture and serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, and albumin were tested. Histopathology of liver sections stained with H & E histological sectioning was prepared according to conventional methods.

FINDINGS: In diabetic rats, increase in serum liver enzymes, showed significant damage compared to the control group ($p \leq 0.05$). While in the diabetic group treated with milk containing LA, these indicators when compared to diabetic group was significantly lower ($p \leq 0.05$). The lowest level of ALT, AST and ALP in diabetic rats treated with 20% milk containing LA was 78 ± 6.5 , 205 ± 3.4 and 168 ± 1.9 , respectively. Histopathologic findings were in agreement with the results of serum biochemical markers confirmed the repair of hepatic damage in the diabetic rats after intervention.

CONCLUSION: This study showed that milk containing LA has a protective effect on the liver of rats with streptozotocin-induced diabetes.

KEY WORDS: Diabetes, Hepatic, *Lactobacillus Acidophilus*, Liver enzymes, Streptozotocin.

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* Corresponding Author; A. Ghorbani Ranjbary(DVM)

Address: Young Researchers and Elite Club, Kazerun Branch, Islamic Azad University, Kazerun, I.R.Iran

Tel: +98 71 42243921

E-mail: dr_alighorbani87@yahoo.com

Introduction

Different genetic factors leading to types I and II diabetes mellitus. Both of them are prone to complications such as nephropathy, retinopathy, peripheral nerves disorders, and blood pressure (2 and 1). Diabetes is as the leading causes of hepatic disorders in the United States of America. In many studies have shown that liver disease is an important cause of morbidity and mortality in type II diabetes (3). Beside, the high prevalence of liver disease in diabetic patients, the incidence of diabetes is higher in patients with liver disease. It seems that the incidence of liver disease in diabetes type II occurs due to complications such as abnormal liver enzymes, non-alcoholic fatty liver, cirrhosis, hepatocellular carcinoma and acute liver failure is more prevalent. (4).

In patients with type II diabetes, mild increase in the transaminase enzymes is more common, in addition, the palliative interventions such as hemodialysis or retinal photocoagulation is not available for hepatic complications of diabetes. Thus, despite the hepatic complications of diabetes are less common, but they are as threatening as complications like *Glomerulopathy*, retinopathy and nephropathy. Annual check-ups to determine the liver diseases can be made by simple laboratory tests such as serum alanine aminotransferase level (5). Oxidative stress has recently been implicated as one of the mechanisms of diabetes and its relevant complications (6). Free radicals are routinely produced in interactions in the body as a result of normal metabolic processes, and environmental stimulus. In physiological conditions in the living species, various antioxidant defensive mechanisms act against the harmful effects of free radicals (6).

Oxidative stress results from an imbalance between the production of active oxygen species (ROS) and antioxidant factors that promote cleansing and removal of ROS. For example, an increment or decrease in production of ROS and antioxidant factors or both can lead to oxidative stress. In diabetes, protein glycosylation and glucose oxidation can produce free radicals, which will catalyze lipid peroxidation (7, 8). Furthermore, defects in the antioxidant defense system such as changes in antioxidant enzymes (9) glutathione metabolism disorders (10), and decreased level of ascorbic acid has been shown in diabetes (12, 11).

Until now, the increased oxidative stress in living species has not been clearly approved. Nevertheless, several studies on human and animal models using Thiobarbituric Acid Reactive Substances (TBARS)

method have shown increase in lipid peroxidation in

membranes and lipoproteins in diabetes (18-13). Feillet-Coudray *et al.* studied the liver antioxidant status and lipid peroxidation in drug-induced diabetes (19). In another study, it has been shown that anti-diabetic agents can reduce levels of serum biomarkers, representing the liver damage (20). But these factors also can cause severe side effects (21). In recent years, the desire to use beneficial bacteria so called probiotics as diet supplements has been investigated in the food industry (22). The adequate intake of probiotics, have had beneficial effects on the normal microbial population of the gastrointestinal tract. The most commonly used probiotic microorganisms are *Lactobacillus acidophilus* and *Bifidobacterium Bifidum* (23). Based on animal and human studies, probiotics act in the multiple levels for regulation of reduction of the endogenous inflammatory mediators.

The main pathways are the regulation of the immune system, modulating intestinal bacterial flora, improving tight junctions of the intestinal wall, reducing the permeability of pathogens into the blood circulation and anti-fibrosis and anti-inflammatory activities (24). By now, there is no definitive medical treatment for non-alcoholic fatty liver disease (NAFLD). The efficacy of probiotics in several experimental models of NAFLD/NASH is approved.

In a study on ob/ob mice treated with high-fat diet, in this experimental model of obesity, the alteration of the intestinal flora was affective on the fatty liver disease. The VSH#3 probiotic improves hepatic parenchyma, by reducing total fat and serum levels of liver alanine aminotransferase (ALT) (25).

In another study, VSH#3 probiotic could improve the lipid profile and reduce the inflammation, oxidative damage and the tissue levels of TNF- α (26). So far, few studies have examined the effect of probiotics in the treatment of NAFID in human study. In another study, the daily consumption of probiotic supplements for three months, have decreased the serum levels of liver enzymes (ALT, AST and gamma- glutamyl transpeptidase (GGT)) (27).

Due to the beneficial effects of probiotics and their ability to lower the levels of liver enzymes and glucose levels, their impact on improving the status of the liver in diabetes, the effectiveness of the milk contains LA is not unexpected. The aim of this study was to investigate the protective effect of the milk containing LA in the streptozotocin-induced diabetic rats.

Methods

This experimental study was conducted on 60 male Wistar rats weighing 220-190 g, by which were randomly divided into 6 groups including: control, diabetic, healthy rats treated with 20 and 10 percent of milk containing LA, diabetic rats treated with 20 and 10 percent of milk containing LA, respectively.

To produce milk containing LA, according to McFarland method, LA inseminated to rats in treated groups. Daily, in average, in groups treated with 10% milk containing LA, 10^6 CFU bacteria was consumed by each rat and in 20% groups, the amount of 10^{12} CFU bacteria were consumed by each rat (28). Animals to adapt to the new environment, prior to the beginning of the study, caged for one week at 25 ° Celsius with 12 hrs light and 12 hrs darkness. The rats of all groups for 60 days plus water received milk (25% of total liquid).

For induction of diabetes, a single dose of 65 mg/kg streptozotocin (Sigma Co. Tehran, Iran) was injected intraperitoneally. Animals after 18 hours fasting, with blood glucose level greater than 16.5 mmol were considered diabetic and were used in this study (29). Since the start of the experiment, rats fed with milk containing LA for 60 days by 10 and 20% of water. After 60 days, fasting blood samples were obtained directly from the rat heart and was centrifuged in speed of 2500 rpm for 15 min at 30 ° C (EBA21 model, Hettich, Germany) and serum was isolated from cells. Then the serum biomarkers of liver function, including

ALT, AST, ALT, total bilirubin, and albumin (33-30) were measured using commercially available kits (Pars test kits). After killing the mice by high doses of anesthetic substance (ketamine), rats' liver was removed and biopsies obtained and then after stabilization in 10% buffered formalin and embedded in paraffin, sections with 5 microns thickness was prepared and stained with hematoxylin-eosin to prepare histopathologic sections. Optical microscope (E200 model, NIKON ECLIPSE) was used to evaluate the histological changes.

Using SPSS software version 17 for statistical analysis, ANOVA test including ad Hoc tests like Tukey's, Sheffe and Duncan were measured. In all measurements P-value less than 0.05 was considered statistically significant.

Result

The serum levels of ALT, ALP, AST and total bilirubin in the diabetic groups compared with the control group was significantly higher ($p \leq 0.05$). Factors listed in the treated diabetic groups were significantly lower compared to control diabetic group ($p \leq 0.05$). The lowest level of liver enzymes (ALT, ALP and AST), was measured in diabetic rats receiving 20 percent milk containing LA compared to other diabetic groups (78 ± 6.5 , 205 ± 3.4 and 168 ± 1.9 , respectively) ($p \leq 0.05$) (table 1).

Table 1. Comparison of the effect of milk containing LA on serum markers of liver damage in studied rats

Research groups	AST(U/L) Mean±SD	ALP(U/L) Mean±SD	ALT(U/L) Mean±SD	Total bilirubin (Mg/dl) Mean±SD	Albumin (g/dl) Mean±SD
Control group	3.2±160	3.5±190	2.4±76	0.04±0.82	0.32±4.38
Diabetic groups	2.7±220	3.2±275	5.4±148	0.06±1.16	0.25±3.12
Healthy group treated with 10% milk containing LA	3.2±154	5.4±205	1.2±78	0.07±0.8	0.4±4.3
Healthy group treated with 20% milk containing LA	5.2±148	2.3±201	2.3±75	0.07±0.79	0.56±4.3
Diabetic rats treated with 10% milk containing LA	3.6*±170	4.3*±219	3.1*±79	0.05*±0.86	0.3*±4.15
Diabetic rats treated with 20% milk containing LA	1.9*±168	*3.4±205	*6.5±78	*0.02±0.84	0.35*±4.29

- Each value represents the mean ± standard error of the mean plasma levels of ALT, AST, ALP (U/L)

- Each value represents the mean ± standard error of the mean plasma concentration of albumin (g/dl)

- Each value represents the mean ± standard error of the mean plasma concentrations of total bilirubin (Mg / dl)

Sign asterisk * indicates a significant difference ($p \leq 0.05$) between diabetic groups treated milk containing Lactobacillus and diabetic control group

Albumin levels in the diabetic group was significantly lower compared with the control group and the this factor in diabetic rats treated with 20 and 10 percent milk containing LA compared to diabetic group was significantly higher ($p \leq 0.05$). Treatment milk containing LA in the healthy group showed no significant change compared with the control group, though, caused a mild decrease in the liver enzymes (ALT, ALP and AST), and the total bilirubin. Liver pathology assessment in the control group showed healthy and normal liver tissue (fig 1). Also, in the control group treated with 20 and 10 percent of milk containing LA, no pathological changes in liver tissue was made. But in the diabetic group certain fatty changes was made in centrilobular areas (fig 2). Again in the liver of diabetic groups that treated with 20 and 10 percent of milk containing LA no specific pathological changes were observed (fig. 3 and 4).



Figure 1. Microscopic view of a rat liver tissue of the control group. The structure is completely healthy (hematoxylin-eosin stained magnification 40X)

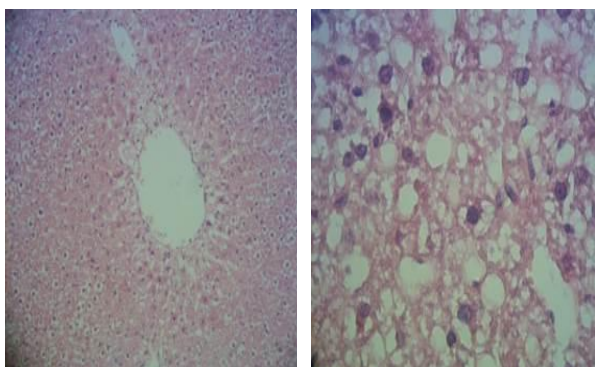


Figure 2. Microscopic view of a rat liver tissue of diabetic rats. Fatty change in liver hepatocytes in central Lobules of the liver with Micro-vesicles accumulation of fat (hematoxylin-eosin stained magnification 40X and 100X)

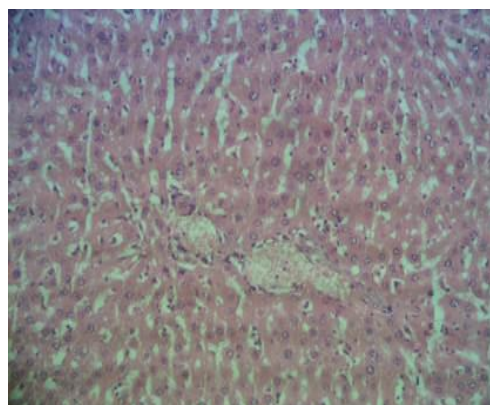


Figure 3. Microscopic view of a rat liver tissue of diabetic rats received 10% of milk containing Lactobacillus with healthy liver tissue structure (hematoxylin-eosin stained magnification 40X)

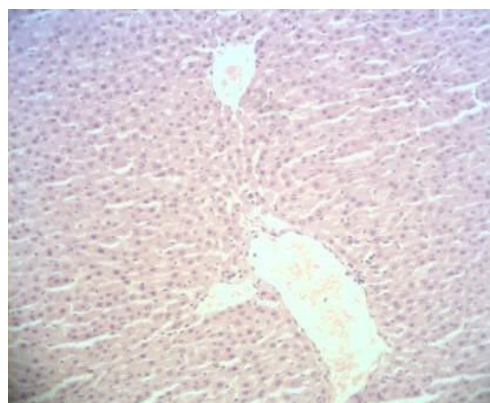


Figure 4. Microscopic view of a rat liver tissue of diabetic rats receiving 20% of milk containing LA. The structure is completely healthy liver tissue (hematoxylin-eosin stained magnification 40X)

Discussion

In this study, a significant decrease in serum albumin levels and increase in markers of liver injury (ALT, ALP and AST) were observed. These results are corresponding with the findings of Rammesh and colleagues in 2007 (35). Liver function abnormalities are more prevalent in the patients with type II diabetes (20) that is consistent with our results. The present findings also showed that daily consumption of milk containing LA considerably improves the biochemical and histopathologic condition of liver parenchyma in the diabetic rats.

Disruption of the integrity of the plasma membrane of hepatocytes release the liver function enzymes that are naturally present in the cytosols, to the bloodstream (36). Streptozotocin toxin in addition to destructive

effects on pancreas beta-cells is harmful for other organs such as liver. Also, it has damaging effects of oxidative stress on the liver which reduce the amount of albumin in diabetes induced subjects (38 and 37). This decrease indicates that the liver is unable to synthesize these factors (37).

Most animal studies on NAFLD, have reported that probiotics can improve the factors associated with NAFLD and inflammatory processes such as NF- κ B activity and secretion of TNF- α (39, 38). Li and colleagues also studied the effect of probiotic VSL # 3 in experimental models of fatty liver disease induced by fat-enriched diet and reported that the same as TNF- α antibody, VSL#3 has an improving effect in the histology of liver by reducing the amounts of fatty acid of the liver and ALT serum levels (25), which confirms the results of other mentioned studies. Histological analysis shows the expression of fatty changes in centrilobular parts of liver tissue in diabetic rats. Rammesh and colleagues in 2007, reported the benefits of protecting liver cells in diabetic rats induced by streptozotocin, that their results are consistent with our findings (35). In our study, the consumption of milk

containing LA in diabetic rats did not show any significant changes in fat, showing the protective effect of this type of milk against liver complications of diabetes. Also, the pathological findings in this study are consistent with results obtained biochemically.

Overall, in this study we found out that milk containing LA reduce the level of serum biomarkers of liver damage and so reduce the organ function damage in streptozotocin-induced diabetic rats. Our results showed that milk containing LA has protective effects on the liver in diabetes induced by streptozotocin. Since mechanisms of action it is not well known, more studies especially on TNF- α and the protective mechanisms of milk containing LA are suggested.

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